Def: Using RE to digest DNA (plasmid, PCR product)

2 purposes:

1) Cloning: cut and paste DNA
   - Need a lot of DNA, large reaction volume (50 microliters)

2) Checking: check if our plasmid is correct
   - Better checking method compared to colony PCR
   - Need less DNA, small reaction volume (10 microliters)

2 Rules for Restriction Enzyme digestion

1) Restriction Enzymes are proteins, sensitive to heat, always keep them on ice or ice-block
2) Restriction Enzymes usage should always <10% of total reaction volume

Protocol:

Cloning

1)

Substance	Amount
DNA	1-5 micrograms
10X buffer	5 µL
Restriction Enzyme 1	1 µL
Restriction Enzyme 2	1 µL
d2H20	X µL
Total	10 µL

2) Incubate at 37˚C (for most enzymes) for more than 1 hour - overnight

3) Gel electrophoresis

Checking

1)

Substance	Amount
DNA	200ng - 1 micrograms
10X buffer	1 µL
RE 1	.4 µL
RE 2	.4 µL
d2H20	X µL
Total	10 µL

2) Incubate at 37 ˚C (for most enzymes) for more than 30 minutes
3) Gel electrophoresis